THE RADIOSENSITIVITY OF EGGS OF ARBACIA PUNCTULATA IN VARIOUS SALT SOLUTIONS[1]

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A variety of experimental procedures has been shown to alter the sensitivity of cells to x-rays and radium. Resistance to radiation can be increased by a reduction of oxygen (Crabtree and Cramer, 1933; Mottram, 1935; Anderson and Turkowitz, 1941); by the use of appropriate concentrations of ammonia (Zirkle, 1936; Marshak, 1938); CO_2 and H_2S (Zirkle, 1936, 1940, 1941) and by addition of protein to the medium in which the cells are immersed (Evans et al., 1941). Conversely, certain agents increase the radiosensitivity of biological material (see Scott, 1937). The present study has been carried out to ascertain whether alteration of the salt environment, which will in turn cause changes in the ionic composition, and to some extent the colloidal state of the protoplasm, will influence the action of x-radiation on the living cell.

Three solutions have been used to alter the ionic composition of the egg: isotonic potassium citrate; a mixture of isotonic $MgCl_2$ and sea water; and a mixture of isotonic $CaCl_2$ and sea water. Potassium citrate is of particular interest in this connection in that it will remove a large part of the calcium from the cell and at the same time is relatively non-toxic. A further point of interest lies in its inhibition of the reactions initiated by ultra-violet light in the Nereis egg (Heilbrunn and Wilbur, 1937). Magnesium, like citrate, is inhibitory with respect to ultra-violet action (Wilbur, 1939). Calcium is antagonistic to both citrate and magnesium in many reactions of living material and so has been studied along with these two ions in the present work.

Methods

Prior to irradiation 0.1 to 0.2 cc. of concentrated eggs was added to 40 cc. of the experimental solution or sea water for various periods. The eggs were then transferred to small plastic dishes for irradiation. Following irradiation 0.15 to 0.25 cc. of solution containing the irradiated eggs was placed in 250 cc. of sea water to remove the experimental solution; and approximately 6 minutes later the eggs were transferred to a second dish of sea water which contained sperm. The time required for 50 per cent of the eggs to complete first cleavage was determined by fixing samples at 2-minute intervals in 1 per cent or 2.5 per cent formaldehyde in sea water after examination of the eggs showed that cleavage had begun. In a few instances in which the cleavage time occurred very slowly

[1] A grant from the Graduate School of The Ohio State University is gratefully acknowledged.
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samples were fixed at 3-minute intervals.[4] By this method one can estimate the time to 50 per cent cleavage in normal eggs within one or two minutes. After very large doses of x-rays many of the eggs show multipolar cleavage, and it is not always easy to decide the exact time at which the cleavage furrows have cut completely through the egg. In such cases determinations of the time of cleavage are accordingly somewhat less accurate. In most experiments the

TABLE I

Effect of x-radiation on Arbacia eggs following treatment with 0.35 M potassium citrate

Exp. No.	Cleavage time of non-irradiated eggs			Cleavage time of eggs receiving 30,400 r			Cleavage time of eggs receiving 53,200 r		
	Eggs in sea water throughout	Eggs treated with potassium citrate		Eggs in sea water throughout	Eggs treated with potassium citrate		Eggs in sea water throughout	Eggs treated with potassium citrate	
		For 30 min.	For 60 min.		For 30 min.	For 60 min.		For 30 min.	For 60 min.
I	II	III	IV	V	VI	VII	VIII	IX	X
1.	45	43					207	166	
2.	41	42					118	110	
3.	41	43					148	130	
4.	42	42					175	150	
5.	44	43					176.5	152	
6.	44	42					159	147	
7.	45	44					208	186	
8.	43.5	59					174	159	
9.	44	43		172.5	171				
10.	42	41.5		136	138				
11.	45	43		128	124				
12.	44	46		145	140*				
13.	39	39		188	186				
14.	43.5		42	121		108	147		122
15.	41		42	120		122	152		139†
16.	50		49	141		124	164		161†
17.	44		47	128		124	155		138
18.	45		45	146		140	166		151

* Total cleavage 76%–79%.
† Total cleavage 85%–86%.

percentage of multipolarity was estimated for the control and experimentally-treated eggs. Only those batches of eggs were used which on fertilization showed well-lifted membranes on at least 95 per cent of the eggs. During treatment with experimental solutions and x-radiation the eggs were at room temperature, which varied from 21 degrees to 26 degrees. Fertilization and cleavage were carried out in a water bath at a temperature of 25.01 ± 0.06 degrees.

The following solutions were used: 0.35 M potassium citrate; $CaCl_2$-sea-water mixture consisting of two parts of sea water and one part 0.3 M $CaCl_2$; and a $MgCl_2$-sea-water mixture made up of equal parts of 0.3 M $MgCl_2$ and sea water. The calcium content of the $CaCl_2$-sea-water mixture is approximately 9.6 times

[4] A very few times the small numbers of available eggs made it necessary to make counts on the living eggs.

that of sea water. The $MgCl_2$-sea-water mixture has a magnesium content 3.3 times that of sea-water. The pH of sea water was 7.9, and the pH of all experimental solutions was 7.6 ± 0.2.

The x-radiation was carried out with the dual tube self-rectifying outfit available at the Marine Biological Laboratory. The secondary voltage was 182 kv., and the current on each tube was 25 ma. The distance from the center of each target to the center of the material irradiated was 9.5 cm. The eggs were irradiated in small plastic dishes approximately 2 cm. in diameter. The depth of the solution containing the eggs was approximately 0.9 cm. Experiments 1 through 8 (Table I) were carried out at an output of 7,600 r per minute, while all other experiments were exposed at an intensity of 5,600 r per minute.

Viscosity was determined by means of an Emerson hand centrifuge at a centrifugal force of approximately 1960 × gravity (Wilbur, 1940).

Results

Experiments with Potassium Citrate

Cleavage Time

Unfertilized eggs treated with 0.35 M potassium citrate for 30 and 60 minutes were given various doses of x-rays and returned to sea water within 30 seconds following irradiation. The well known effect of roentgen rays in delaying the

Table II

Effect of x-radiation on Arbacia eggs treated with potassium citrate for 20 minutes prior to and 20 minutes following irradiation

Exp. No.	Cleavage time of non-irradiated eggs		Cleavage time of eggs receiving 15,200 r		Cleavage time of eggs receiving 30,400 r	
	Eggs in sea water	Eggs treated with potassium citrate for 40 minutes	Eggs in sea water	Eggs treated with potassium citrate	Eggs in sea water	Eggs treated with potassium citrate
1.	45 min.	45 min.	82 min. (for 63% cl.)	82 min. (for 63% cl.)	110 min.	103 min.
2.	46 min.	46 min.	91	87	128	125
3.	43	42	72	70	126	120
4.	45	47	82	81	125	117
5.	45	45	79	66	138	108
6.	45	45	115	102	170	154
7.	46	46	Exovates on nearly all. Poor cleavage	No exovates. 98% cleavage	Exovates on nearly all. Poor cleavage	Exovates rare. 100% cleavage
8.	43	43	79	78	128	116

cleavage time is shown in Table I. With a dose of 30,400 r the eggs which had been in potassium citrate for 60 minutes cleaved somewhat sooner than the sea-water controls in four of the five cases (columns V and VII). With 53,200 r in 12 of the 13 cases studied the citrated eggs cleaved several minutes sooner than those in sea water (columns VIII, IX and X); and the 30-minute citrate treatment was quite as effective here as the 60-minute treatment. Smaller doses of 3,800

and 15,200 r delay cleavage to the same degree in citrated treated eggs and eggs in sea water (not shown in table).

Eggs treated with potassium citrate for 20 minutes prior to the completion of irradiation and allowed to remain in citrate for 20 minutes following irradiation were also protected from the x-ray action to some degree. The effect is clear-cut with 30,400 r and is indicated in some cases at 15,200 r (Table II). Although a 30-minute treatment with citrate prior to and during x-radiation has little or no protective action for a dose of 30,400 r (Table I, columns V and VI) a 20-minute treatment prior to and during x-radiation and followed by an additional

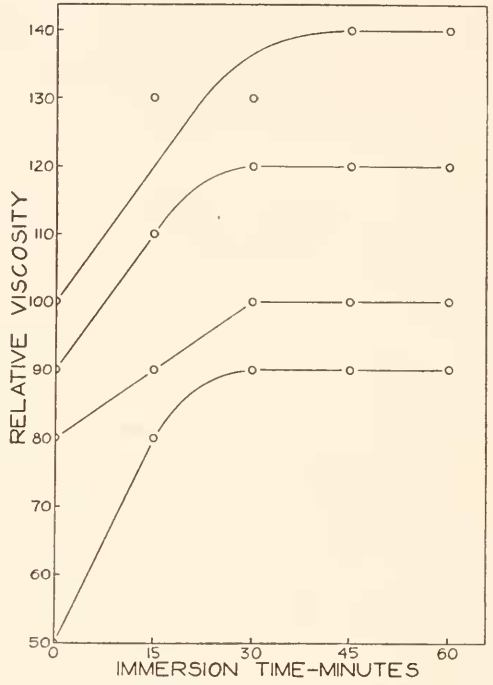

FIGURE 1. Viscosity of unfertilized Arbacia eggs in 0.35 M potassium citrate. The relative viscosity (ordinates) was measured following treatment in potassium citrate for various periods. (abscissas). pH 7.6 Temperature 24.0–25.2° C.

20-minute immersion after irradiation may inhibit the x-ray action. The difference is not especially striking, and we should not care to stress the point on the basis of the evidence at hand. However, the data do suggest the interesting possibility that the x-ray effect can be inhibited somewhat by changing the ionic composition of the protoplasm *following* the period of irradiation.

When eggs are x-rayed in sea water and immersed in potassium citrate immediately afterward, the citrate has no protective action. Five such experiments were carried out in which eggs were given doses of 15,200 and 30,400 r and changed from sea water to citrate in less than 30 seconds following irradiation, and immersed for 30 minutes. In this case some time would be required for equilibrium to be established between the citrate and the egg; and reactions

initiated by the radiation may have gone to completion before the citrate exerted its full effect.[5]

Viscosity

The effect of 0.35 M potassium citrate on the colloidal state of the Arbacia egg at the time of irradiation as reflected in its viscosity has been studied. The viscosity changes of eight batches of eggs have been determined, and the results for four of these are shown in Figure 1. It is to be noted that potassium citrate causes an increase in viscosity. The highest value is usually reached in 30 minutes and maintained constant with continued immersion. Our concern has not been with the mechanism of the viscosity increase produced by potassium citrate. However, it may be pointed out that the potassium ion will in itself increase the viscosity of protoplasm (Heilbrunn, 1937). Mazia (1940) has found a marked decrease in the calcium content of Arbacia eggs treated with potassium citrate; and this has been confirmed by Miss Pauline Hamilton for the particular conditions of our experiments.

Experiments with Magnesium Chloride

Experiments similar to those with potassium citrate were carried out with a mixture of equal parts of 0.3 M $MgCl_2$ and sea water. The total period of immersion in the experimental solution was 60 minutes. The response to x-radiation of eggs treated with this mixture was much the same as in sea water. In each of six experiments doses of 15,200 r and 30,400 r were used. A dose of 53,200 r was employed in four experiments.

Viscosity determinations on eggs immersed for 55 minutes in the $MgCl_2$-sea-water mixtures revealed a slight decrease in seven of nine batches of eggs. The average decrease in viscosity for these seven samples was approximately 12 per cent.

Experiments with Calcium Chloride

The effects of x-radiation on eggs treated for 60 minutes with a mixture of one part 0.3 M $CaCl_2$ and two parts sea water were similar to those produced on eggs irradiated in sea water. Doses of 3,800 r, 15,200 r, 30,400 r and 53,200 r were used.

The $CaCl_2$-sea-water mixture resembles $MgCl_2$-sea-water mixture causing a slight decrease in the viscosity of unfertilized eggs. The average decrease for five batches of eggs was about 15 per cent after 60 minutes treatment.

The Viscosity of Unfertilized Eggs Following X-Radiation

In collaboration with Mr. Walter Wilson the viscosity of unfertilized Arbacia eggs has been studied after irradiation in sea water in order to ascertain whether roentgen rays will produce viscosity changes in the living cell. A dose of 30,400 r was employed and the viscosity determined 25 minutes following the completion

[5] Such an assumption, however, involves an apparent contradiction in that the possible enhanced effect resulting from leaving eggs in citrate for a 20-minute period following irradiation would argue that the x-ray effect was not complete shortly after irradiation. But the situation in which sea water replaces citrate is not necessarily comparable to the present one in which citrate replaces sea water.

of the irradiation or approximately 30½ minutes from the time that irradiation was begun. The viscosity determinations were carried out at 24.4–25.8° C. This dosage has a drastic effect upon cleavage. The average cleavage time for 21 experiments was 134 minutes as compared with 44 minutes for the non-irradiated control eggs. The majority of eggs receiving this dosage also exhibit multipolar cleavage. However, this relatively enormous dose failed to produce detectable changes in the viscosity of the egg (five experiments).

The centrifuge method as used here would enable one to distinguish between a relative viscosity of 70 units and one of 60 units, for example. Our negative results therefore apply only to differences of this order of magnitude.

We are indebted to Dr. L. V. Heilbrunn for his co-operation and advice; to Miss Pauline Hamilton for her kindness in carrying out the calcium analyses; and to Dr. F. R. Hayes for helpful suggestions during the preparation of the manuscript.

Discussion

The data presented indicate that potassium citrate inhibits the effect of x-radiation on cell division. However, the inhibition is slight and appears only with high x-ray doses. The effect of the citrate treatment prior to irradiation is to increase the viscosity of the protoplasm and to reduce the calcium content. But it is also almost certainly true that immersion of a cell in potassium citrate upsets the entire ionic equilibrium of the cell and not merely the calcium content. In view of this, the influence of the potassium citrate treatment may involve substances other than calcium. Dale (1942) found that various substances, including sodium oxalate, sodium nitrate and sodium nitrite, would inhibit the destruction of d-amino-acid oxidase by x-rays. As yet, however, there is no justification for assuming enzyme inhibition by citrate in the case of the Arbacia egg.

The experiments with calcium-rich and magnesium-rich sea water together with the citrate experiments at lower x-ray doses indicate that the egg probably can tolerate a considerable change in ionic composition without an alteration in radiosensitivity. That the colloidal state of the protoplasm was affected by the addition of these ions is shown in most cases by a change in the viscosity which is increased by potassium citrate and decreased by sea water containing excess calcium or magnesium.

Experiments were described pointing to a possible action of potassium citrate after the period of irradiation. Even in those cases in which eggs were changed from citrate to sea water immediately following irradiation, some time would be required before equilibrium could be established. It may be true that the entire action of citrate is exerted after irradiation. If such is the case, one would have to assume that at least a portion of the x-ray action is indirect. That is, the x-radiation initiates a reaction which is partially inhibited in the citrated egg.

It is rather remarkable that the viscosity of the unfertilized egg is unchanged by doses of radiation which so greatly alter the rate and normal course of cell division. The direct coagulation of proteins as an explanation of the biological effects of roentgen rays would seem to be ruled out in the present study (see Zirkle, 1940).

We should like to suggest that the chief action of x-rays on the egg is the alteration of some system, perhaps enzymic, which comes into prominence after fertilization and is of particular importance for certain phases of mitosis. This explanation has also been suggested for colchicine which may be without effect on the viscosity of the unfertilized Arbacia egg, yet changes the viscosity of the fertilized egg and inhibits cell division (Wilbur, 1940). That radiation may interfere with cellular respiratory systems has been pointed out by several workers (see, for example, Crabtree and Cramer, 1933; Rudisill and Hoch, 1938).

We may call attention to the interesting fact that eggs can be treated for relatively long periods with isotonic potassium citrate or solutions of high calcium or magnesium content and yet on return to sea water they can be fertilized and will usually cleave at a normal rate. The citrate and magnesium treatments may, however, cause a slight amount of multipolarity.

Summary

1. Treatment of Arbacia eggs with 0.35 M potassium citrate inhibited the retarding action of x-radiation on cell division. However, the inhibition by citrate was slight and appeared mainly with high x-ray doses (30,400 and 53,200 r).

2. The radiosensitivity of the egg was unaffected by increasing the calcium or magnesium content of the sea-water medium.

3. The potassium citrate treatment employed increased the viscosity of the unfertilized egg. The viscosity was decreased slightly in the sea-water solutions of increased calcium or magnesium content.

The data presented indicate that changes in the ionic composition and viscosity of the protoplasm may occur without altering the sensitivity of the egg to x-radiation.

4. Doses of x-radiation which markedly altered the rate and normal course of cell division produced no detectable change in the viscosity of the unfertilized egg.

LITERATURE CITED

Anderson, R. S., and H. Turkowitz, 1941. The experimental modification of the sensitivity of yeast to roentgen rays. *Amer. Jour. Roent.*, **46**: 537–541.

Crabtree, H. G., and W. Cramer, 1933. The action of radium on cancer cells. II. Some factors determining the susceptibility of cancer cells to radium. *Proc. Roy. Soc. London*, B, **113**: 238–250.

Dale, W. M., 1942. The effect of x-rays on the conjugated protein d-amino-acid oxidase. *Biochem. Jour.*, **36**: 80–85.

Evans, T. C., J. C. Slaughter, E. P. Little, and G. Failla, 1941. The influence of the medium on the radiosensitivity of sperm. *Biol. Bull.*, **81**: 291–292.

Heilbrunn, L. V., 1937. *An Outline of General Physiology.* W. B. Saunders Co., Philadelphia: 76–77.

Heilbrunn, L. V., and K. M. Wilbur, 1937. Stimulation and nuclear breakdown in the Nereis egg. *Biol. Bull.*, **73**: 557–564.

Marshak, A., 1938. Alteration of chromosome sensitivity to x-rays with NH_4OH. *Proc. Soc. Exp. Biol. Med.*, **38**: 705–713.

Mazia, D., 1940. The binding of ions by the cell surface. *Cold Spring Harbor Sympos.*, **8**: 195–203.

Mottram, J. C., 1935. On the alteration in the sensitivity of cells towards radiation produced by cold and by anaerobiosis. *Brit. Jour. Radiol.*, **8**: 32–39.

Rudisill, H., and J. H. Hoch, 1938. How x-rays may kill cells. *Radiol.*, **31**: 104–106.

Scott, C. M., 1937. Some quantitative aspects of the biological action of x and γ rays. *Med. Res. Counc. Spec. Rep. Ser.*, No. 223.

Wilbur, K. M., 1939. The relation of the magnesium ion to ultra-violet stimulation in the Nereis egg. *Physiol. Zool.*, **12**: 102–109.

Wilbur, K. M., 1940. Effects of colchicine upon viscosity of the Arbacia egg. *Proc. Soc. Exp. Biol. Med.*, **45**: 696–700.

Zirkle, R. E., 1936. Modification of radiosensitivity by means of readily penetrating acids and bases. *Amer. Jour. Roent.*, **35**: 230–237.

Zirkle, R. E., 1940. The influence of intracellular acidity on the radiosensitivity of various organisms. *Jour. Cell. Comp. Physiol.*, **16**: 301–311.

Zirkle, R. E., 1941. Combined influence of x-ray intensity and intracellular acidity on radiosensitivity. *Jour. Cell. Comp. Physiol.*, **17**: 65–70.